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ORIGINAL PAPER

Effect of Transient Spark Discharge and Plasma Activated Water Treatments against *Fusarium graminearum* Infected Wheat Grains under Laboratory Conditions

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Abstract
Over the last decade, more and more attention has been paid to applications of non-thermal plasma in agriculture, where it is used to decontaminate various microorganisms and to improve the seed germination. In this study, we present the results of a newly developed point-to-ring NTP transient spark discharge apparatus (NTP), plasma activated water (PAW) and their combined treatment on Durum wheat and Common wheat grains under laboratory conditions. Transient spark discharge treatment was used as direct treatment while indirect treatment of wheat grains was performed by PAW produced in point-to-plane NTP transient spark apparatus. We found that the degree of grain surface decontamination was in order NTP > PAW > combined treatment. In the case of Durum wheat grain germination, all treatments increased germination with increasing exposure times, while in the case of Common wheat, PAW treatment and combined treatment did not significantly increase the grain germination. In conclusion, plasma treatment has enormous potential for use in agriculture and its possibilities need to be fully explored.

Keywords *Fusarium graminearum* · Wheat grain · Transient spark · Plasma activated water · Surface decontamination · Grain germination

Introduction

Agriculture is one of the most important occupations in the world. Wheat is one of the most important commodities and second most cultivated crop [1]. According to the Food and Agriculture Organization of the United Nations (FAO) data, there is an increase of 1.0% in cereal production in 2023, but wheat output is predicted to fall below 2022 levels, while the FAO expects 0.7% increase in wheat consumption [2]. For decades, wheat production is suffering globally due to different pests and pathogen infections. One of the most disastrous pathogens that infects cereals, and especially wheat, is *Fusarium graminearum*, also known by the name *Gibberella zeae*. It is the causal agent of Fusarium head blight (FHB), a

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devastating disease on wheat. Traditional agricultural practices are unable to overcome this disease and this pathogen is known to decrease yield significantly. Moreover, this pathogen produces mycotoxins causing vomiting and liver failure, thus posing a threat in both agriculture and food industry [3], for more details, see [4–7].

Decades of research has been dedicated to control this pathogen using various control strategies; yet the control of this phytopathogen remains a challenge. Several chemical fungicides were researched and adopted against this pathogen. Yet, it has over the years, developed resistance against different chemical fungicides such as benzimidazoles, triazoles, carbendazim, as reviewed by de Chaves [8]. Development of resistance to chemical fungicides and due to their unsustainable nature, finding alternative methods to chemical control that are sustainable and environment friendly, have recently gained significant importance.

Non-thermal plasma (NTP) is described as a partially or fully ionized gas made up of different ions, molecules, and reactive species; its where overall temperature usually does not exceed 40 °C [9]. NTP has several advantages over conventional treatments, such as short treatment time, easy accessibility, and low temperature during operations Attri et al. [10] and is therefore widely used in many different fields, including medicine [11]. Different feeding gases alter plasma chemistry leading to variations in the treatments [10]. Recently, a lot of attention is given to the use of NTP in agriculture, focusing mainly on the seed's surface decontamination. Apart from decontamination properties, NTP is also studied to study seed surface changes, germination of seeds etc. Scholtz et al. [9], for which the collective term "Plasma agriculture" is being coined by Ranieri et al. [12]. The success of NTP on seed surface decontamination and seed germination depend on the right conditions to which the seeds are exposed; any excess exposure to NTP can also have detrimental effects on the seeds [13].

The plasma activated water (PAW) may be prepared by exposing water or other medium to different types of discharge [9, 14, 15]. It has been used as an inflexibility of NTP devices to decontaminate seed surface uniformly [16]. PAW contains several short-lived and long-lived components such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) [16]. The short-lived reactive species such as OH radicals, singlet oxygen, peroxynitrite are very difficult to measure as they quickly react upon production with other reactive species in the medium, whereas long-lived species such as hydrogen peroxide, nitrates and nitrites can be measured easily. This is why according to Scholtz et al. [9], the active particles content in PAW is lower as compared to the direct plasma discharge. The measurement of long-lived reactive species can be found in the research done by Julák et al. [17].

Combining different NTP treatments is a novel area of research. Xu et al. [18] tested sequential combination NTP treatment followed with PAW treatment against *Saccharomyces cerevisiae* and *Aspergillus flavus*. Our team has experience with the decontamination of artificially infected seeds with the *Fusarium circinatum* and *F. oxysporum* through the direct action of NTP [19, 20]. In this study, we tested the decontamination of *F. graminearum*, which is pathogenic mainly on cereals [3]. The aims of this research were to test the time-dependent disinfection efficiency of newly developed point-to-ring transient spark NTP apparatus against *F. graminearum* on two wheat species. Additionally, we also tested efficiency of a combined treatment i.e., newly developed point-to-ring transient spark discharge NTP treatment along with PAW against *F. graminearum* in the two wheat species.

Materials and Methods

Point-to-ring NTP Transient Spark Discharge Apparatus and its Characteristics

Scheme of the NTP-producing apparatus used for seeds treatment and discharge characterization is shown in Fig. 1. It is based on an atmospheric pressure DC driven discharge operating in a self-pulsing transient spark mode. The discharge is generated in a point-to-ring electrode arrangement in ambient air at atmospheric pressure and room temperature. The point electrode connected to the positive terminal of the DC high voltage power supply is made of stainless-steel needle with a diameter of 1.8 mm and with symmetrical tip of curvature radius 0.3 mm. The ring electrode connected to the negative terminal of the DC high voltage power supply is made of a ferrite magnet in the shape of an annulus with an inner diameter of 25 mm, an outer diameter of 40 mm and a height of 6 mm. The distance between the tip of the point electrode and the upper edge of the ring electrode is 11 mm. Transient spark discharge burns between the tip of the point electrode and the ring electrode, the reactive particles created by the non-thermal plasma are blown by the electric field through the ring electrode down onto the treated seeds as shown below in Fig. 2.

Samples to determine the concentration of reactive species NO, NO_2 and O_3 were taken by the PTFE tube of inner diameter 3 mm placed under the ring electrode over the exposed seeds. NO and NO_2 concentrations were measured by Serinus 40 H NO_x analyser (ACOEM Ecotech) using chemiluminescence detection; NO and NO_2 range was up to 1000 ppm and the measurement accuracy 1%. Ozone concentration was measured by UV-100 Ozone Analyzer (Eco Sensors), which measures absorption of the 254 nm UV spectral line; the measurement range was 0.01–999 ppm, accuracy 2%.

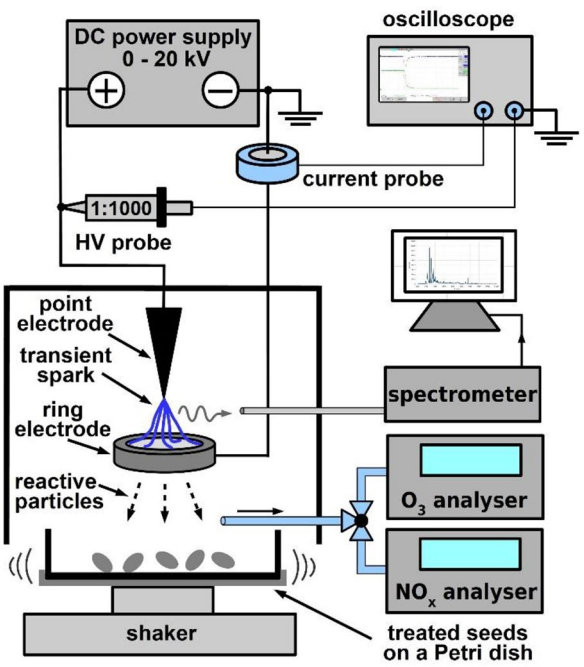

Fig. 1 Schematic of point-to-ring NTP transient spark discharge treatment apparatus used in this study

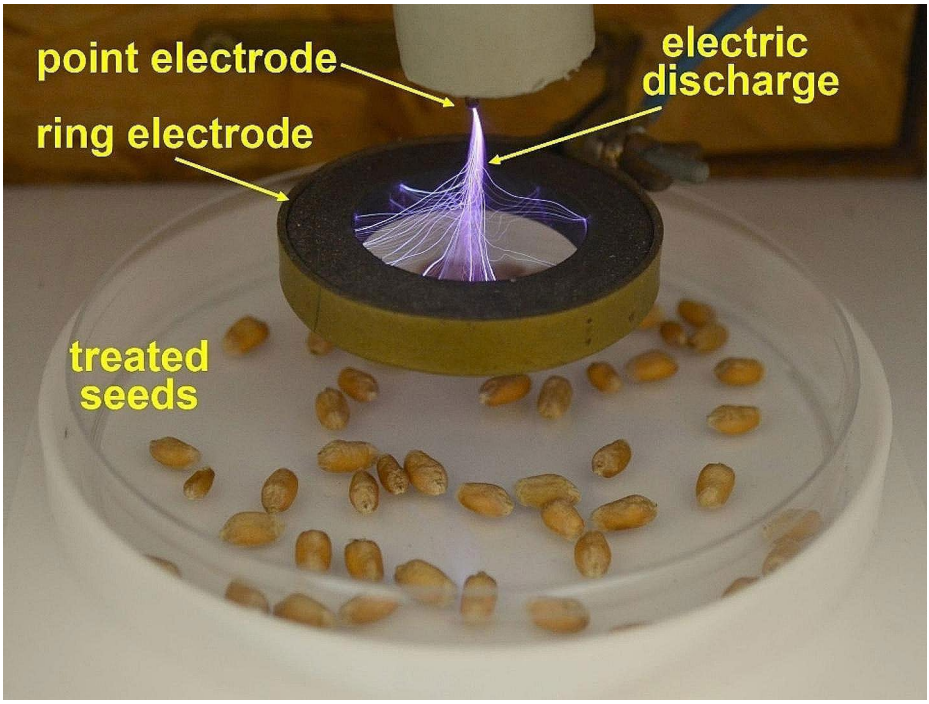

Fig. 2 Treatment of wheat grains using point-to-ring NTP transient spark discharge apparatus. The Petri dish is placed on a shaker as shown in the schematic diagram

Emission spectra of used electrical discharge in the region of 200–1100 nm were measured using an HR2000+spectrometer (Ocean Optics) with a charge-coupled device (CCD) detector. The discharge radiation was fed into the spectrometer by a solarized optical fiber focused by fused silica collimating optics. The spectralresolution of the spectrometer measured as full width half maximum is 0.65 nm. Communication with the spectrometer enabled the computer program SpectraSuite.

Voltage waveform of the discharge was measured using HV probe, CT4028, input impedance 900 MΩ, divider ratio 1000:1, bandwidth 220 MHz (Cal Test Electronics, Inc.). Current waveform was measured using current probe - Wide Band Current Transformer, model 2877, bandwidth 200 MHz, sensitivity 1 V/A (Pearson Electronics, Inc.). Both probes were connected to digital oscilloscope T3DSO1302A (Teledyne Lecroy), bandwidth 350 MHz, sample rates up to 2 GSa/s.

Preparation of PAW

PAW was prepared using atmospheric pressure DC driven discharge operating in a self-pulsing transient spark mode in point-to-plane electrode geometry as used by Jirešová et al. [21] with a slight modification. Instead of exposing one millilitre of distilled water for 30 min, we exposed 4 mL of distilled water for 2 h. Point-to-plane transient spark discharge was supplied by 9 kV DC power supply, through ballast impedance realised by the parallel connection of 20 MΩ resistor with 120 pF capacitor. The ground electrode was a platinum

wire immersed in the water of which surface represented the plane electrode; point electrode was made by a stainless steel medical needle. The distance between the point electrode and the surface of the water was adjusted to approx. 2–3 mm so that an average current of 300 μA was obtained. The arrangement is schematically shown in Fig. 3. The procedure was repeated several times to obtain sufficient amount of PAW which was stored in glass bottles covered in aluminium foil with lid to minimize interaction of PAW with air and light. Electrical and optical characteristics of the discharge were presented in previous work (Khun et al. [22]) and the issue of PAW generation and its characterization in Hozák et al. [23].

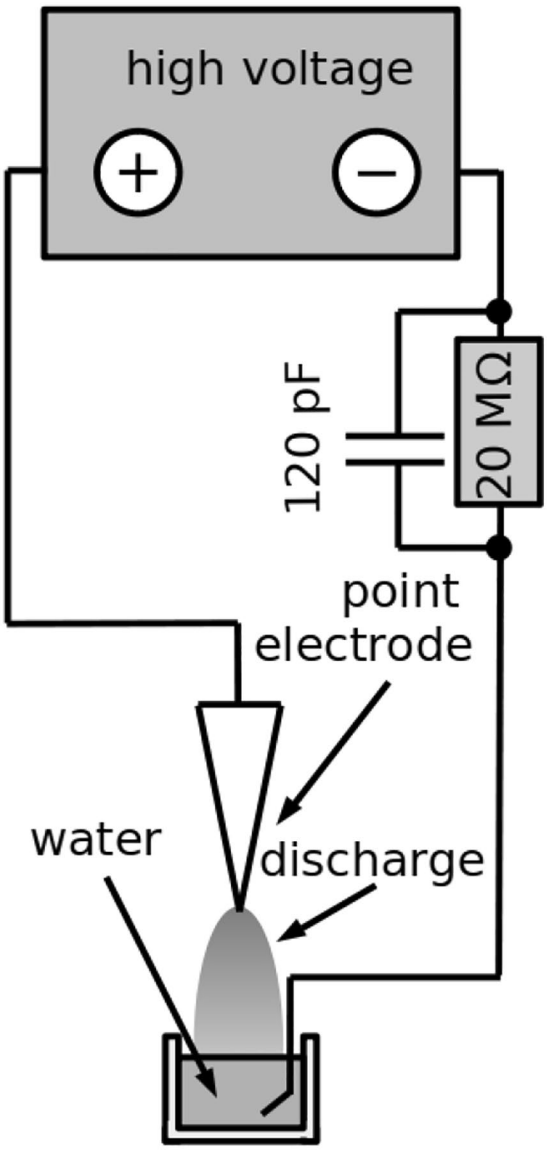

Fig. 3 Scheme of the point-to-plane transient spark apparatus used for generating of PAW

Chemical Analysis of PAW

Due to our previous results in Jirešová et al. [21], we considered that PAW contains mainly hydrogen peroxide (H_2O_2) and nitrogenic acids as reactive particles. Hydrogen peroxide was determined by manganometric titration and by the semiquantitative test strips QUANTOFIX Peroxide 100. The total nitrogenic acids content was determined by alkalimetric titration using NaOH, the particular ones were estimated by the QUANTOFIX Nitrate test strips.

Source of Grains and Fungus

Two wheat species, Common wheat (*Triticum aestivum* L. var. Frisky and Durum wheat (*Triticum Durum* Desf.) of organic quality, were used for this experiment. Grains of Common wheat) were obtained from Osivo Boršov (93% seed germination, 50 g weight of thousand seeds), whereas grains of Durum wheat were obtained from commercial suppliers in the Czech Republic (95% germination, 52 g weight of thousand seeds). Only whole and undamaged grains were sorted and used for the experiment.

Fusarium graminearum strain (DM4224) was obtained from the collection of Yeasts and Industrial Microorganisms of the Department of Biochemistry and Microbiology at the University of Chemistry and Technology, Prague. It was sub-cultured on Maltose extract agar (MEA) plates under sterile conditions and kept in the incubator in dark conditions at 23 °C for growth for 10 days. For the experiment, conidial solution was prepared in sterile distilled water and the concentration was adjusted to of 1×10^6 using Bürker chamber. This concentration of conidial suspension was already severe, like other studies, ensuring each wheat grain is exposed to heavy conidial infection.

Experimental Procedures with Grains

A total of 150 grains per treatment were used for each experiment, divided into 30 grains aliquots for 5 replicates. The grains surface was sterilized using 5% sodium hypochlorite (NaOCl) for 30 s. After draining the NaOCl solution, the grains were dried for 30 min on filter paper in laminar airflow box. Grains were then inoculated by immersing in *F. graminearum* conidial solution for 30 min. Then, the conidial solution was drained, and the grains were dried for 10–15 min on filter paper in laminar airflow box. The grains were then subjected to different treatment. The infected control was performed with grains infected by *Fusarium graminearum* conidial solution, while the negative control was performed with seeds immersed in sterile distilled water. The details of the treatments are summarized in the Table 1.

For the treatment nos. 9 and 10 as mentioned above in the table, we used the NTP treatment for 1 min followed by PAW treatment for 24 h. In our pilot study, we found > 70% of the wheat grains still infected after NTP 1-min treatment. Therefore, we decided to combine the NTP 1-min treatment along with PAW treatment, since PAW already consisted of H_2O_2 and NO^{3-}. Additionally, we wanted to avoid over-exposure of grains to the plasma treatment as adverse effects were observed by Mildaziene et al. [24] while Henselová et al. [25] reported the adverse effects in maize. In case of the NTP treatments nos. 3, 4, 5, 6, 9, 10 thirty seeds were exposed to the NTP in Petri dish placed on a shaker (see Fig. 2). Imme-

Table 1 Summary of the treatments on both wheat varieties. The order of infecting and treatment of wheat grains were followed in the sequence as shown in the table below

Sr. No	Abbreviation of the treatment	Description of the treatment
1	DW	Grains treated with distilled water (negative control)
2	Fus	Grains artificially infected with *Fusarium graminearum* (infected control)
3	NTP 3 min	Grains treated with NTP for 3 min without *Fusarium graminearum*
4	Fus + NTP 3 min	Grains artificially infected with *Fusarium graminearum* followed by NTP treatment for 3 min
5	NTP 5 min	Grains treated with NTP for 5 min without *Fusarium graminearum*
6	Fus + NTP 5 min	Grains artificially infected with *Fusarium graminearum* followed by NTP treatment for 5 min
7	PAW	Grains treated with PAW for 24 h without *Fusarium graminearum*
8	Fus + PAW	Grains artificially infected with *Fusarium graminearum* followed by Plasma activated water treatment for 24 h
9	NTP 1 min + PAW	Grains treated with NTP for 1 min followed by PAW for 24 h without *Fusarium graminearum*
10	Fus + NTP 1 min + PAW	Grains artificially infected with *Fusarium graminearum* followed by treatment with NTP for 1 min followed by PAW for 24 h

diately after the treatment, the seeds were checked for temperature using FLIR E4 thermal imaging camera. After treatment, the seeds were transferred into Petri dishes containing sterile filter papers and six millilitres of distilled water were poured on it. All operations were performed under sterile conditions. The Petri dishes were incubated for 7 days in dark at 23 ± 0.5 °C. Surface grain infection was confirmed by eye as a visible mycelium growth, the germination was confirmed as the occurrence of radicle from the grain.

Statistical Analysis

All data were analysed using the R statistical software at the significance level of 0.05. The testing of experimental variances among each other was done using the one-way ANOVA test followed by the Tukey HSD test for multiple comparisons or by an independent t-test for two comparisons. Data were presented as mean ± SD in tables whereas in graphs as grain infection (%) against time period was plotted; both supplemented by different letters indicating significant difference at 95% confidence level.

Results

Point-to-ring Transient Spark NTP Apparatus Characteristics

The NTP source used is based on an atmospheric pressure DC discharge operating in a self-pulsing transient spark mode with a pulse repetition rate of about 2.5 kHz. A photograph of the discharge is shown in Fig. 4. The current during the discharge pulses reached 5 A with the full width at half maximum of ~ 50 ns (Fig. 5). The instantaneous power of the discharge reached 70 kW in a pulse. The energy deposited into the discharge per pulse was ~ 4 mJ, and the mean discharge power was approximately 10 W. The surface temperature of the seeds did not exceed 50 °C.

The time-integrated emission spectrum of the discharge is shown in Fig. 6. The spectrum is dominated by the second positive system of the nitrogen molecule. The first positive system of the nitrogen molecule was also present in the spectrum, but its intensity was an order of magnitude lower than the second positive system. The emission of the negative molecular nitrogen system was very weak and difficult to detect. The emission of hydroxyl radical as well as atomic hydrogen, which can be formed by a discharge in humid air, were not registered. The emission spectrum clearly shows the presence of atomic oxygen and nitrogen in the plasma, which indicates a high degree of dissociation of oxygen and nitrogen molecules. This is explained by the release of relatively high instantaneous energy into a narrow discharge channel. In addition we measured the concentrations of nitrogen oxides and ozone formed in the non-thermal plasma source. The concentration of nitric oxide (NO) was 40 ppm, nitrogen dioxide (NO_2) was 15 ppm, and ozone (O_3) was 0.2 ppm.

Chemical Characteristics of PAW

The concentration of H_2O_2 in PAW was 250 ± 15 mg/L.

The concentration of NO^-_3 in PAW was 1210 ± 60 mg/L. These results were also confirmed by the Quantofix Peroxide 100 and Nitrate strips, which also confirmed the absence of NO_2^- ions.

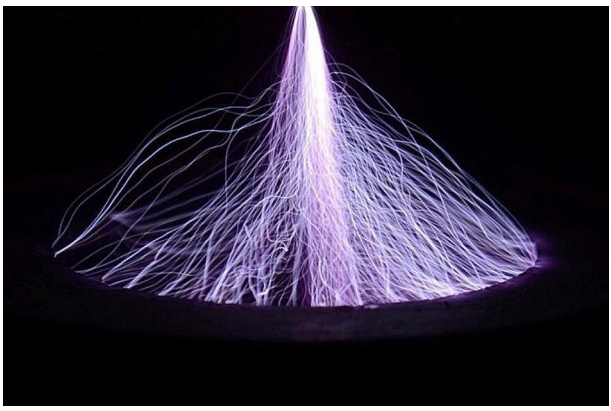

Fig. 4 Appearance of the point-to-ring transient spark discharge produced by the NTP source

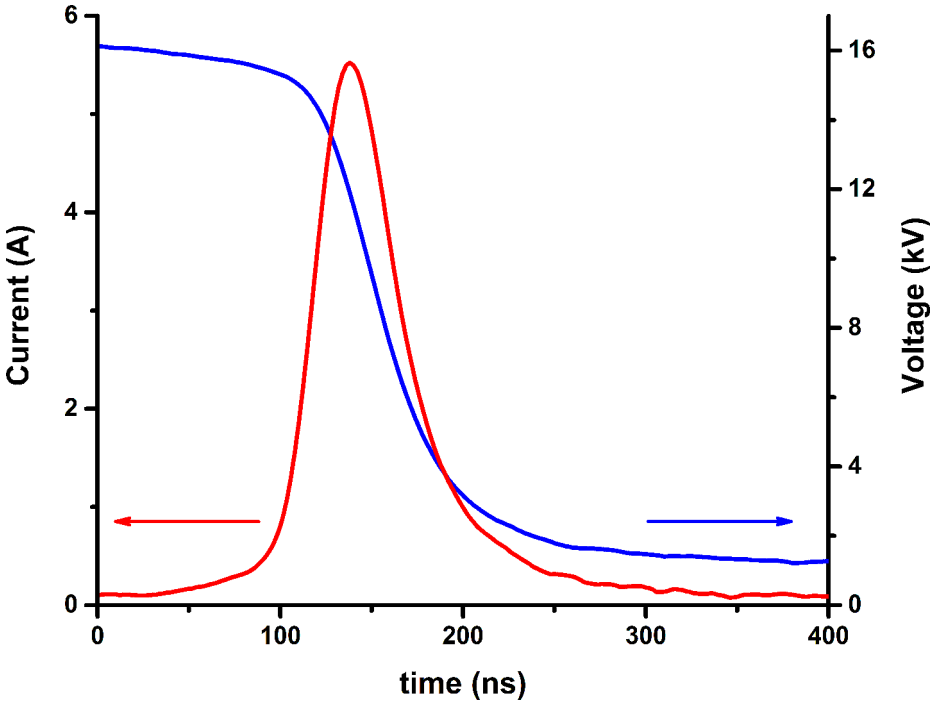

Fig. 5 Waveforms of the discharge voltage (blue line) and current (red line)

Experimental Procedure with Grains

For the sake of convenience, the results of two wheat varieties are presented in separate subsections, i.e., (a) Durum wheat and (b) Common wheat.

Durum Wheat

In case of Durum wheat, grains treated with [Fus+NTP 3 min] and [Fus+NTP 5 min], a significant reduction of infected seeds against the infected control was observed (Fig. 7). Until day 7, it is evident that the treatment [Fus+NTP 5 min] significantly lowers percentage of infected seeds (<50%) as compared to the infected control while the treatment [Fus+NTP 3 min] showed less than 60% infected seeds. PAW treatment [Fus+PAW] was also effective and the infected seeds were significantly lower as compared to infected control until Day 5 (Fig. 8). Until Day 3, there were less than 30% of infected seeds. It was also observed that the efficacy of PAW faded after Day 5.

We also investigated a combined treatment of seeds with two different treatments, i.e., [Fus+NTP 1 min+PAW] (Fig. 9). It was found out that seed infection was significantly lowered until Day 3 with infected seeds being less than 40%, then the effectiveness of the treatment weakened until it completely disappeared.

The grain germination was observed and recorded daily until Day 7. The Table 2 displays results for different treatment and Durum wheat germination. Our interest is to understand

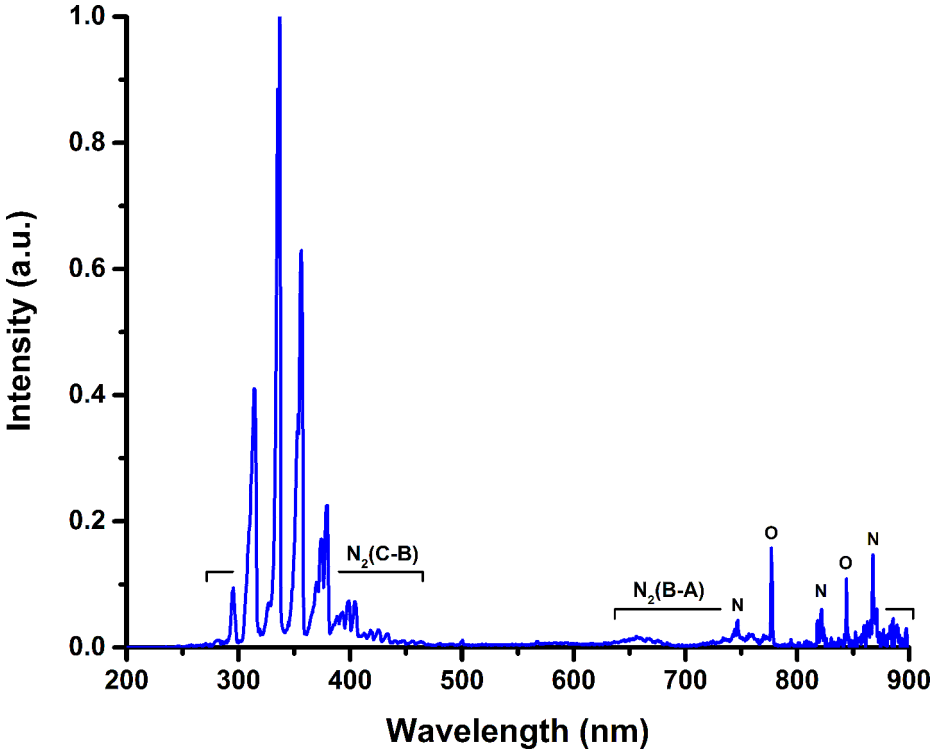

Fig. 6 Time-integrated emission spectrum of the discharge

how grain treatments infected with *F. graminearum* performed against their respective control, DW treatment (negative control) and Fus treatment (infected control) daily until Day 7.

In the case of treatments [Fus + NTP 3 min] and [Fus + NTP 5 min], from Day 2 onwards, significant enhancement in Durum wheat grain germination was observed with almost 90% germination on Day 4 as compared to [Fus] control and were comparable with the negative control treatment [DW]. For [Fus + PAW] treatment, a significant enhanced germination was observed only from Day 6 (> 70%) as compared to [Fus] control treatment. It was however less effective as compared to [PAW] and [DW] control treatments. The Durum wheat germination in the combined treatment of [Fus + NTP 1 min + PAW] was found to be in line with its control treatment [NTP 1 min + PAW] and also [DW] control treatment, achieving > 80% germination after Day 3. It is evident that all the treatments showed an increase in germination with increasing exposure time. This indicates that NTP, PAW and the combined treatment had a positive effect on the seed germination despite being infected with *F. graminearum* and in their respective treatment control.

Common Wheat

The treatments [Fus + NTP 3 min] and [Fus + NTP 5 min] showed more prominent results of seeds disinfection as compared to the Durum wheat. Both, the 3-min and 5-min treatment with the NTP, significantly restricted the *Fusarium graminearum* growth at all time period.

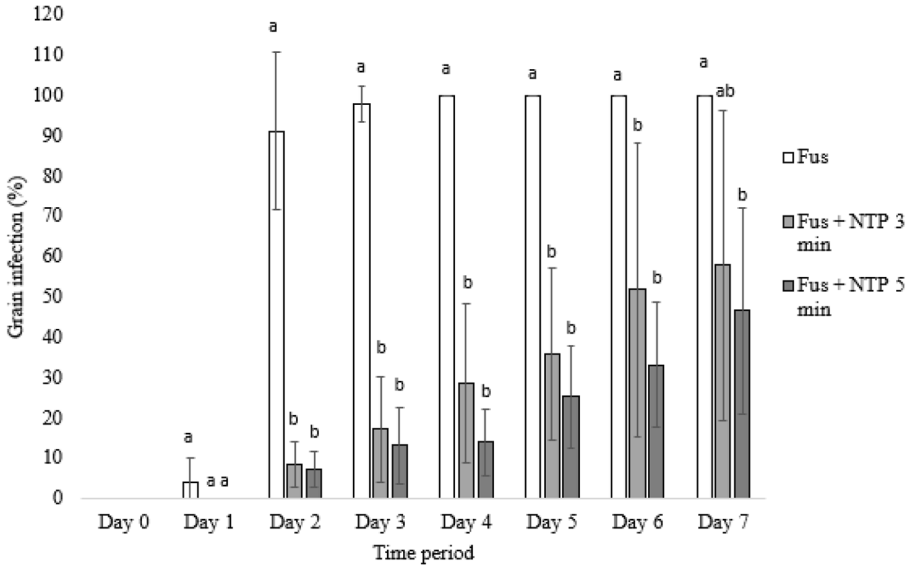

Fig. 7 Efficacy of treatment [Fus+NTP 3 min] and [Fus+NTP 5 min] on Durum wheat grains infected with *Fusarium graminearum*. ANOVA post-hoc Tukey test. Different letters on top of the error bars indicate significant difference at 95% confidence level

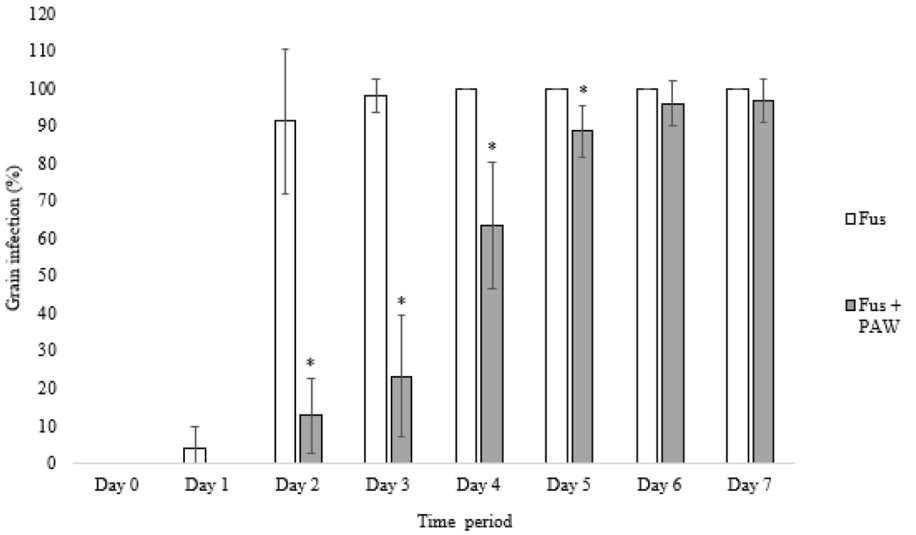

Fig. 8 Efficacy of treatment [Fus+PAW] on Durum wheat grains infected with *Fusarium graminearum*. Independent t-test was performed on the data. '*' sign on top of the error bars indicates significant difference at 95% confidence level

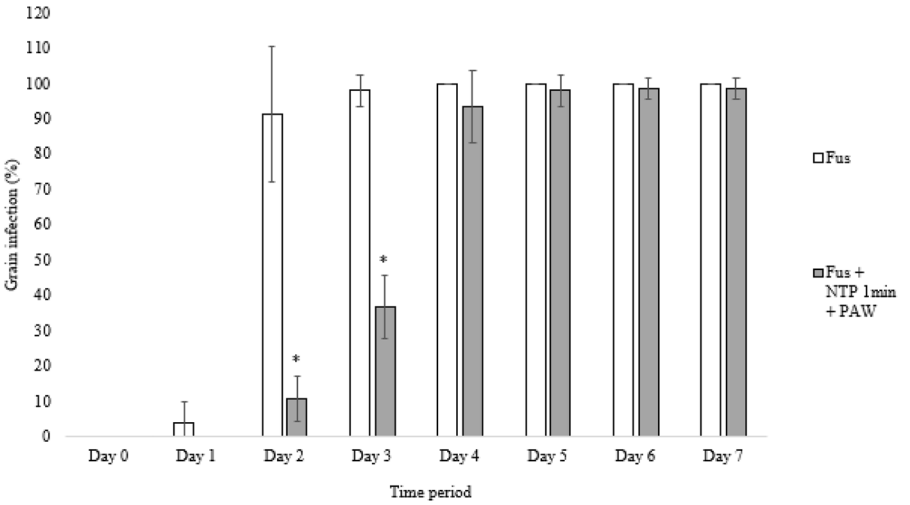

Fig. 9 Efficacy of treatment [Fus+NTP 1 min+PAW] on Durum wheat grains infected with *Fusarium graminearum*. Independent t-test was performed on the data. '*' sign on top of the error bars indicates significant difference at 95% confidence level

Table 2 Germination of Durum wheat grains (percentage±SD) recorded for 7 days after various treatments

Treatments	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DW	27±5 ab	87±cd	92±4 b	96±5 c	97±4 c	97.0±2.8 c	97.0±2.8 c
NTP 3 min	30±9 ac	90±7 d	93±7 b	96±5 c	98.0±1.8c	97.0±2.8c	99.0±1.5c
NTP 5 min	18±8 a	84.0±2.8 cd	93±5 b	95±3c	97±4 c	97.0±2.8c	99.0±1.5c
NTP 1 min+PAW	72±8 e	93±5 d	95±3 b	97.0±2.8c	99.0 ± 1.8c	99.0±1.5c	99.0±1.5c
PAW	60.0±2.4 de	88±6 cd	93±5 b	95±4 c	97.0±2.4c	97.0±2.8c	98±3 c
Fus	33±20 ac	47±15 a	53±20 a	53±21 a	53±21 a	53±21 a	54±21 a
Fus+NTP 3 min	51±12 ce	83±12 cd	86±9 b	87±11 c	90±10c	90±10bc	91±10bc
Fus+NTP 5 min	39±13 acd	82±7 cd	89±8 b	91±5 c	92±6 c	93±4 bc	94±4 bc
Fus+NTP 1 min+PAW	47±10 bcd	68±17 bc	81±5 b	81±5 bc	82±6 bc	82±6 bc	82±6 bc
Fus+PAW	51±8 ce	55±9 ab	63±5 a	65±9 ab	67±10ab	76±14 b	77±14 b

Different letters following SD represent statistically significant difference at 95% confidence level

In fact, at the end of the experiment, i.e., on Day 7, the number of infected seeds did not exceed 20% as compared with [Fus] control treatment (see Fig. 10).

The treatment with PAWon Common wheat grains showed some effect, but not as pronounced as [Fus+NTP 3 min] and [Fus+NTP 5 min] treatments. On Day 3 (approximately 70%), Day 4 (approximately 80%) and Day 5 (approximately 90%) seeds were found to be infected as compared with [Fus] control treatment (Fig. 11). Again, the activity of the plasma activated water faded as the time increased, which resembles the same trend as observed in Durum wheat.

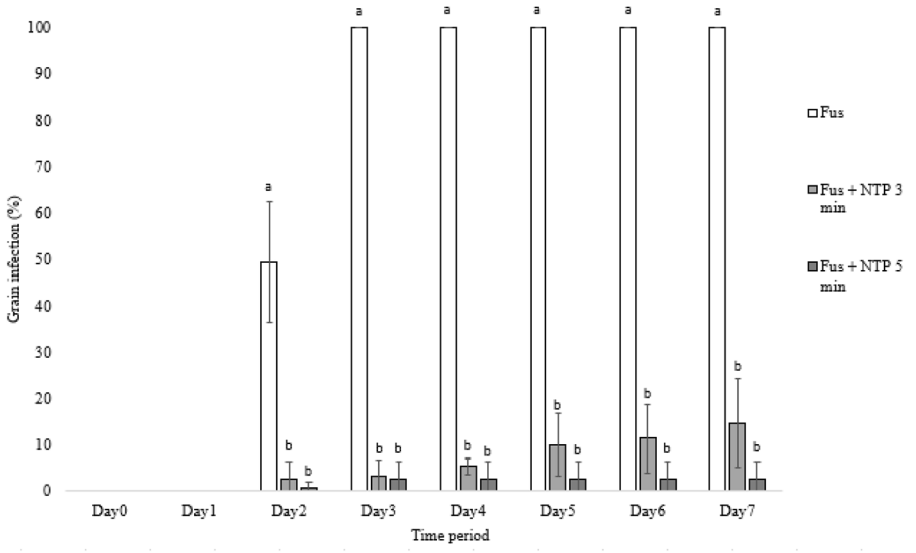

Fig. 10 Efficacy of treatments [Fus+NTP 3 min] and [Fus+NTP 5 min] on Common wheat grains infected with *Fusarium graminearum.* ANOVA post-hoc Tukey test was performed on the data. Different letters on top of the error bars indicate significant difference at 95% confidence level

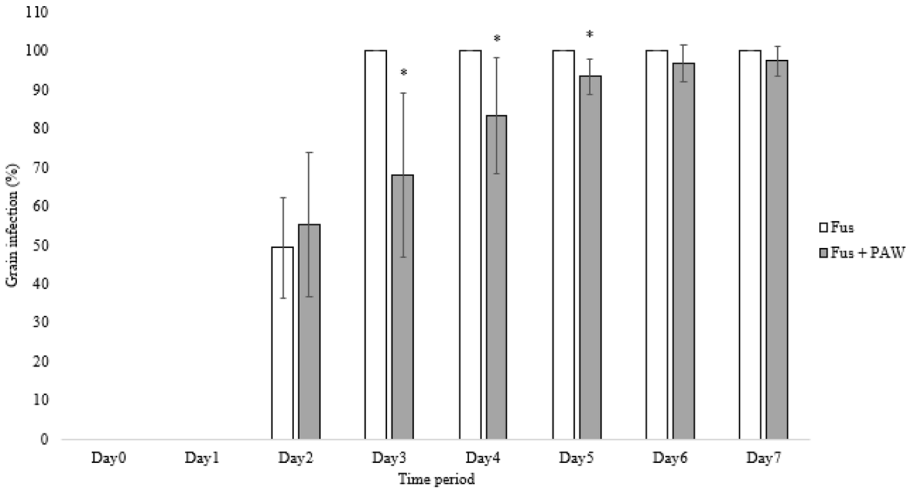

Fig. 11 Efficacy of treatment [Fus+PAW] on Common wheat grains infected with *Fusarium graminearum*. Independent t-test was performed using R statistical software. '*' sign on top of the error bars indicates significant difference at 95% confidence level

The combined treatment [Fus+NTP 1 min+PAW] did not show prominent efficacy except on Day 3 (approximately 80% infected seeds) compared to the control (Fig. 12). Again, it is evident that the efficacy of combined treatment faded as it can also be seen in case of Durum wheat.

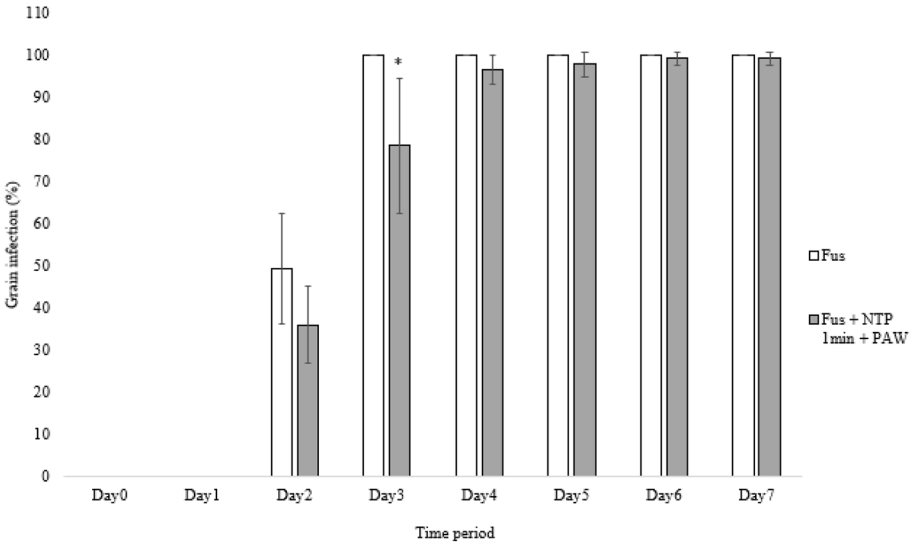

Fig. 12 Efficacy of treatment [Fus+NTP 1 min+PAW] on Common wheat grains infected with *Fusarium graminearum*. Independent t-test was performed on the data. '*' sign on top of the error bars indicates significant difference at 95% confidence level

Table 3 Germination of Common wheat grains (mean(%) ± SD) recorded for 7 days after various treatments

Treatments	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DW	95±5 b	98±3 c	99.0±1.5 d	99.0±1.5 d	99.0±1.5 d	100±0 d	100±0 d
NTP 3 min	85±4 b	97±4 c	100±0 d	100±0 d	100±0 d	100±0 d	100±0 d
NTP 5 min	89±5 b	97±0 c	99.0±1.5 d	100±0 d	100±0 d	100±0 d	100±0 d
NTP 1 min+PAW	17.0±2.8 a	32±9 b	59±6 c	79±14 c	81±15 c	86±7 cd	92±7 cd
PAW	17±7 a	32±4 b	41±6 b	58±5 b	58±5 b	80.0±2.7 c	82.0±1.9 c
Fus	87±5 b	93±5 c	94±5 d	95±5 cd	95±5 cd	95±5 cd	95±5 cd
Fus+NTP 3 min	89±8 b	98.0±1.8 c	100±0 d	100±0 d	100±0 d	100±0 d	100±0 d
Fus+NTP 5 min	83±28 b	88±20 c	97±5 d	97±5 d	98±3 d	98±3 d	99±3 d
Fus+NTP 1 min+PAW	13±5 a	13±6 a	20±10 a	20±10 a	21±11 a	21±11 a	21±11 a
Fus+PAW	20±5 a	27±9 ab	31±10 ab	35±15 a	35±15 a	37±17 b	37±17 b

Different letters following SD represent statistically significant difference at 95% confident levels

In case of Common wheat (see the Table 3), the treatments [Fus+NTP 3 min] and [Fus+NTP 5 min] excellent germination results reaching to almost 100% by Day 3. In case of [Fus+PAW] treatment, less than 40% of the grains germinated as compared to the [Fus] and [DW] control treatments. The combined treatment [Fus+NTP 1 min+PAW] showed the least germinated grains (around 20%) which was significantly much less than its cor-

responding control [NTP 1 min+PAW] and [Fus] and [DW] control treatments. It is evident that [Fus+PAW] and [Fus+NTP 1 min+PAW] was not successful in seed germination when their respective controls were showed a delayed but increase in germination as the time progressed.

Discussion

It is well known that transient spark discharge in air produces predominantly nitrogen oxides and suppresses the formation of ozone [26, 27]. We measured the concentrations of nitrogen oxides and ozone formed in the non-thermal plasma source and found them to be consistent with the above. In PAW, H_2O_2 is produced in gas/liquid interface through two-step reactions as summarized by Lukes et al. [28].

$$e^{-*} + H_2O \rightarrow H\cdot + \cdot OH + e^{-} \quad (1)$$

$$OH + \cdot OH \rightarrow H_2O_2 \quad (2)$$

where e^{-*} is an electron with high kinetic energy. In addition to the H_2O_2, reactive nitrogen species (RNS) such as nitrates and nitrites are also produced by PAW through dissolution of nitrogen oxide formed in air plasma [29]. In our study, we found higher concentration of nitrates while nitrites were in very low concentration and could not be detected. The following reactions shows the generation of nitrates and nitrites in PAW [29]:

$$NO_{2(aq)} + NO_{2(aq)} + H_2O_{(1)} \rightarrow NO_2^{-} + NO_3^{-} + 2H^{+} \quad (3)$$

For the sake of convenience, we sub-divided the discussion of wheat grain decontamination and grain germination into three parts based on the treatments i.e. transient spark discharge NTP, PAW and combined effect of NTP+PAW as outlaid in the result section.

Wheat Grain Decontamination and Germination with Transient Spark NTP

Wang et al. [30] showed that 0.5, 1, 1.5, 2, 2.5, 3, 3.5 min treatment of cold atmospheric plasma generated using dielectric barrier surface micro-discharge generating is efficient in inactivating fungal spores of *F. graminearum* HX01, *F. graminearum* LY26, *Fusarium pseudograminearum* and *Fusarium moniliforme* that are dominant fungal strains causing FHB in vitro and in vivo in wheat grains. We achieved similar result with the newly developed transient spark NTP apparatus after 3- and 5-min treatment (see Fig. 7. and Fig. 10). Different *Fusarium* species were also successfully controlled by different NTP treatments. For instance, Go et al. [31] reported that the non-thermal atmospheric plasma (NTAP) generated in the form of plasma jet, successfully controlled *Fusarium oxysporum* in vitro and in vivo on paprika after 90 s treatment. Similarly, Homa et al. [32], who tested the cold plasma jet treatment against *Fusarium oxysporum* f. sp. *basilici* on basil seedlings, found that the plasma jet treatment showed varying degree of efficiency against this phytopathogen. Our results are also similar to Zahoranová et al. [33] who used Diffuse Coplanar Surface Barrier Discharge apparatus to generate cold atmospheric pressure plasma for 30–300 s to disinfect

Fusarium nivale, *Fusarium culmorum* and other filamentous fungi. This also agrees with some of the previously published results, namely Supakitthanakorn et al. [34], who tested various NTP sources namely non-thermal atmospheric gliding arc, RF corona discharge and dielectric barrier discharge. They found that exposure for 5, 10, 15 and 20 min were effective in disinfecting the seed surface of lettuce infected with *Cercospora lactucae-sativa*. We found similar results with 5 min treatment with our newly developed NTP transient spark apparatus in vivo for both the wheat species (see Figs. 7 and 10).

With respect to grain germination, the Durum wheat grains were more susceptible to the *F. graminearum* infection as compared to the Common wheat (see Table 2). In fact, treatment with NTP alone for infected and non-infected wheat grains showed similar germination indicating that our newly developed point-to-plane transient spark NTP apparatus was successful in reducing the *F. graminearum* conidial load on the grain surface, which is also evident from the grain infection data (see Fig. 7). The Common wheat showed quick germination in the control treatments, though some infection was still observed in the *F. graminearum* control treatment.

Our results with the newly developed point-to-ring transient spark apparatus alone are in line with the study of Dobrin et al. [35], who found that differences in germination of untreated and surface discharged plasma treated wheat grains were not significant. Meng et al. [36] also confirm our results about active impact of NTP on wheat grain germination and seedling growth. Our results also support the investigation of Ussenov et al. [37], who tested effects of NTP on wheat grain germination characteristics and grain surface disinfection. They also found that plasma generated by surface coplanar dielectric barrier discharge apparatus enhanced the growth parameters and increased the enzymatic activity in wheat grains.

Similarly, Iranbaksh et al. [38] treated wheat grains with plasma generated by dielectric barrier discharge apparatus using nitrogen and helium gas and found an interesting observation that there was an initial inhibition of growth of the seeds. However, after one month, the seedlings with inhibited growth grew and were comparable with the control group in terms of enhanced growth rate and biomass accumulation in shoot and leaves. Our results correspond to those reported by Hasan et al. [39], who tested the low-pressure dielectric barrier discharge plasma on the wheat grains. They found that 6 min of the treatment showed an increase in germination percentage in grains as compared to the untreated control while in our case 3- and 5-min treatment was enough to enhance wheat germination. Such enhancement of seed germination using NTP could be attributed to different factors such as modification of seed coat to increase wettability, reactions to different reactive particles in found in the discharge [40].

Wheat Grain Decontamination and Germination with PAW

The efficiency of PAW depends on the duration of seeds exposure to plasma. It can be seen from our results that PAW treatment for 24 h displayed antimicrobial effect to the infection caused by *F. graminearum* conidial solution. Similar results were reported by Ochi et al. [41] where the *Fusarium fujikuroi* infected rice seeds were immersed in sterile distilled water and treated with plasma discharge on surface for 10 min, albeit their mode of treatment was different than our treatment. Our results are also in line with Ju et al. [42] who demonstrated that the PAW prepared by plasma discharge inside the water, reduced the *F. graminearum* spores in vitro after treatments of 30–120 min. The log reduction of fungal spores and

mycelium decreased as the time increased, which we demonstrated from our results with *F. graminearum* conidia. Such results are also supported by Guo et al. [43], who studied the disinfection effects of PAW against *F. graminearum*. They found that the longer exposure and the longer incubation time significantly increased the fungal inhibition ratio, which can also be seen in our results. On the contrary to our results, Feizollahi et al. [7] tested PAW bubbles prepared by various methods against *F. graminearum* and its mycotoxin in barley only to find that the treatment is effective against mycotoxin degradation but not efficient against disinfecting *F. graminearum*. It remains a challenge to understand how long the PAW can show its effectiveness post-treatment of infected seeds. In our research, we demonstrated a time-dependent efficacy of PAW against *F. graminearum* conidia infected wheat grains. Although we found that PAW significantly reduced the infection on wheat grains, its efficacy decreased with time. A possible explanation of the PAW´s efficacy fading could be the reduction in the H_2O_2 and nitrates (NO_3^-) content. The same was confirmed and argued by Wang et al. [30], as they mention that concentration of H_2O_2 less than 400 mg/L in PAW is ineffective in showing antimicrobial effects. Additionally, they confirmed that only 1% efficiency was achieved with 800µM H_2O_2 against yeast cells.

Ju et al. [42] in their study argued that although NO_3^- concentration did not significantly decrease, it could not penetrate the fungal cells, thereby their activity is decreasing. In our study, we immersed the grains in PAW for a period of 24 h and incubated them in dark conditions at 23 °C. We hypothesize that while treatment and incubation process, there is a possibility that the H_2O_2 and NO_3^- interacted with the grains, and H_2O_2 reacted and turned into water, or it could have reacted with air and change to water thereby reducing its efficacy. Our hypothesis is also supported by Suwal et al. [44] and Iwata et al. [45], who stated that reduction in antimicrobial and antiviral activity was observed when PAW was stored at room temperature (20–24 °C) over a period of 20 min to 30 days after preparation. Smet et al. [46] explained that several factors such as test pathogen, different NTP apparatus settings could have an impact on the efficiency of plasma activated liquids. In such case, in the future research, it will be interesting to monitor the concentration of H_20_2 and nitrates in PAW during production, storage and treatment to better determine the efficacy of PAW which will also help in better understanding the mechanism of disinfection on seed surface.

The effects of PAW on seed/grain germination have been extensively studied. Kučerová et al. [47] reported improvement in wheat seedling germination after exposure to PAW, which is also confirmed and reported by Jirešová et al. [21]. In our study, the treatment with PAW showed consistent increase in seed germination with increasing time (see Tables 2 and 3). The effect of PAW 24 h treatment on infected grain germination was more profound in Durum wheat as compared to Common wheat. This could be attributed to the constituents found in PAW i.e., H_2O_2 and NO_3^-. Different PAW generating apparatus with different treatment times have also showed similar effect. For instance, Fan et al. [48] showed that PAW treatment for 15s showed the best germination rate and growth characteristics in mung bean sprouts as compared to the longer treatment times of 30, 60 and 90s. Guragain et al. [49] found that soybean seed germination and seedling growth was enhanced by PAW prepared using dielectric barrier discharge for 20 min. This supports our results that PAW has indeed a positive effect on seed germination. Rathore et al. [50] reported that PAW treatment improved the pea seed germination rate, viability index and mean germination time compared to control and concluded that seed pre-treatment with PAW could improve germination and plant growth. Germination parameters in four seed species, i.e. Buckwheat

(*Fagopyrum esculentum*), Barley (*Hordeum vulgare*), Black Mustard (*Brassica nigra*), and Brown Mustard (*Brassica juncea*), were studied by Guragain et al. [51] using PAW prepared by gliding arc discharge system. They found that all the germination parameters were significantly enhanced in the treated seeds as compared to control. Apparently, our result partially contradicts the study of Feizollahi et al. [7] who found that increase in duration of PAW treatment had a negative impact on barley germination. They argued that one of the possible reasons could be the state of the seeds in which they are used for the experiment, as the effect of PAW could be different for fresh seeds and old seeds.

One of the main problems of PAW apparatus is that it produces the charged particles in a small volume of water. Perhaps the PAW can be potentially replaced by easy chemical mixture of the two chemicals i.e. H_2O_2 and nitrates as investigated by Jirešova et al. [21]. To develop this idea and disseminate it, extensive studies with such artificially/chemically prepared water and testing them on different seeds/grains to better understand their decontamination and germination properties is required.

Wheat Grain Decontamination and Germination with Combined Treatment (NTP + PAW)

As a potentially new and little-explored concept, we used a combination of two variant NTP treatments on pre-infected wheat grains by *F. graminearum* conidial solution, namely the NTP treatment followed immediately by PAW treatment. The rationale behind this concept was to investigate if the combined treatment will show up longer surface decontamination effects on wheat grains. A similar study was performed by Xu et al. [18] who tested effects of sequential cold atmospheric plasma and PAW against *Saccharomyces cerevisiae* and *Aspergillus flavus*. They found that short term NTP treatment followed by continued long term PAW treatment significantly reduced both organisms and was comparable to CAP treatment only. However, their study did not consider the time dependence of the two organisms' growth after the combined treatment. Our results showed significant decrease in infected Durum wheat grains even after Day 3 (72 h) post-treatment while Common wheat did not show any considerable decrease in infected grains (see Figs. 9 and 12 respectively). The possible explanation by Xu et al. [18] was that, first the CAP could disinfect the fungi by the reactive oxygen and nitrogen species (RONS) produced by the CAP, followed by the reactive species present in the PAW, which could destroy the remaining fungal cells. This could potentially open a new area of research in seed disinfection using plasma techniques. Thus, further research in this area of combining two different treatments is required, which may allow understanding of the detailed mechanism of disinfection/decontamination.

We also investigated how effective is the combined treatment on wheat grain germination. From our results, it is evident that in the combined treatment control [NTP 1 min + PAW], the two wheat grain species reacted differently. The new NTP source based on the point-to-ring transient spark in combination with PAW was used for the treatment of two species of wheat infected by the *F. graminearum*. We have found a good results for the increasing of germination in case of Durum wheat grains, but the opposite was observed in case of Common wheat (see Tables 2 and 3 respectively). We hypothesize that this difference in germination of wheat grains could be attributed to genetic makeup of the two wheat grains. Additionally, such difference in germination could because of the stress due to NTP, as it may affect starch and other food reserves stored inside the grain [52].

A similar study of Ji et al. [53] who treated soybean seeds with cold plasma and cultivated them with cold-plasma-activated water but without any fungal infection. They found that biomass and edible quality of soybean sprouts were enhanced. Although in our study, no biochemical analysis on the wheat grains post-combined treatment was performed, the possible effects of such combination of plasma treatments on seed/grain germination needs more research.

In the presence of *F. graminearum*, it can be argued that the RONS present in PAW are interacting first with the *F. graminearum* cells but not killing them completely. We hypothesize that in the combined treatment, NTP 1-min treatment did not disinfect the *F. graminearum* load to the extent as compared with the 3-min and 5-min treatment. Further when these pre-NTP 1-min treated infected grains are introduced in PAW, there is a rapid interaction of the RONS with the rest of the *F. graminearum* conidial load, thereby decontaminating them upon interaction. But at the same time, the RONS could arguably also be interacting with the grain surface, which ceases it from interacting with the other *F. graminearum* conidial load present on the grain surface. Also, there is a possibility that some of the *F. graminearum* conidia enter due to the pre-ruptured seed coat post-NTP treatment, thereby infecting the radicle and eventually the hypocotyl inside the grain. With such interaction, it could possibly be argued that the concentration of the RONS decreases in the following order: PAW > *F. graminearum* interaction on grain surface > interaction with actual grain surface > penetration into the grain. This hypothesized interaction effects can also be confirmed from our disinfection and grain germination data. As we describe earlier, the effect on the germination is like other NTP sources, however, we discussed the advantages and disadvantages of our source from both the laboratory and agriculture points of view. Finally, we postulate new hypothesis about the NTP inactivation of *F. graminearum* and the dependence on the speed of germination.

It is evident that some of the different plasma generating apparatus have similar effects. However, the varying degree of effect, which is seen in one crop/plant species with different NTP generating devices makes them difficult for comparison. Doshi and Šera [3] argued that rather than the design of the apparatus per se, the working conditions such as power, gas used, treatment time and their plasma characteristics should be used for comparative analysis. Based on such comparison, in the future; it could help to compile a list of working conditions irrespective of the apparatus, that can specifically be used for seed/grain treatment for germination and/or decontamination. In this way, after performing further research from laboratory to greenhouse to field trials, it can be recommended for upscaling to an industrial level.

Non thermal plasma is still in an infant stage to be considered as a practical application in agriculture. There exist knowledge gaps in studies with seed/grain decontamination and germination. To make NTP practical, first, the treatment conditions need to be established for treatment of seeds/grains. Further, the treated seeds should be tested for germination after different storage time intervals. This will allow us to understand the degree of efficacy of NTP on seed/grain germination with respect to time. In addition to germination, the seeds can also be tested against different plant pathogens to understand decontamination efficacy of the seeds/grains after different storage time. All of this needs extensive research and development with support of different stakeholders and through legal guidelines. NTP could become one of the possible alternative seed treatment solutions that can replace the harmful chemicals used in seed industry.

Conclusion

The non-thermal plasma can be used in agriculture as a method of surface disinfection of seeds. In this study, we tested a newly developed point-to-ring transient spark discharge NTP apparatus alone and in combination with PAW against *F. graminearum* conidia on two wheat grain species. The fungal growth on wheat grains and wheat grain germination were observed on a day-to-day basis for 7 days post-treatment. The NTP treatment with 3- and 5-min showed excellent grain surface disinfection meanwhile PAW and the combined treatments showed variable results in both the wheat grain species. Grain germination was found to increase post-treatment time until 7 days in both the wheat grain species. The treatments with NTP for 3-min and 5-min showed excellent germination comparable to their respective and negative controls. In case of Common wheat, after treatment with PAW and with NTP for 1 min followed by PAW, germination was significantly lower, but an increase with time could be seen. Possible reasons for low efficacy of PAW could be the loss of reactive agents after interaction with grains and air while treatment. In the case of combined treatment [Fus+NTP 1 min+PAW], it is possible that shorter NTP treatment time (1 min) could not have generated enough reactive species as compared to longer NTP treatment time (3- and 5-min) to decontaminate the grains in the first step followed by presumably decrease in concentration of reactive species in PAW. However, there is no doubt that our newly developed point-to-ring transient spark discharge NTP apparatus alone and in combination with PAW had a positive effect on seed/grain surface disinfection and seed/grain germination as can be seen from our study. The full potential of NTP treatment is yet to be realized and further research into the specifications of plasma sources, their working conditions, the possibilities of different combined treatments need further attention and deeper understanding before it can be upscaled to industrial level.

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Author Contributions PD, BŠ, VS designed the experiments; PD performed the biological experiments, PD performed the statistics on the data, PD wrote the main manuscript; JK, MK, MŠ designed the discharge apparatus, JK, MK investigated the electrical characteristics of the NTP apparatus, JK prepared the PAW apparatus; PD, AO performed the chemical analysis of PAW; PD acquired the funding; JJ reviewed the manuscript. All authors read and approve the manuscript.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare no competing or financial interests to disclose.

Ethical Approval Not applicable.

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